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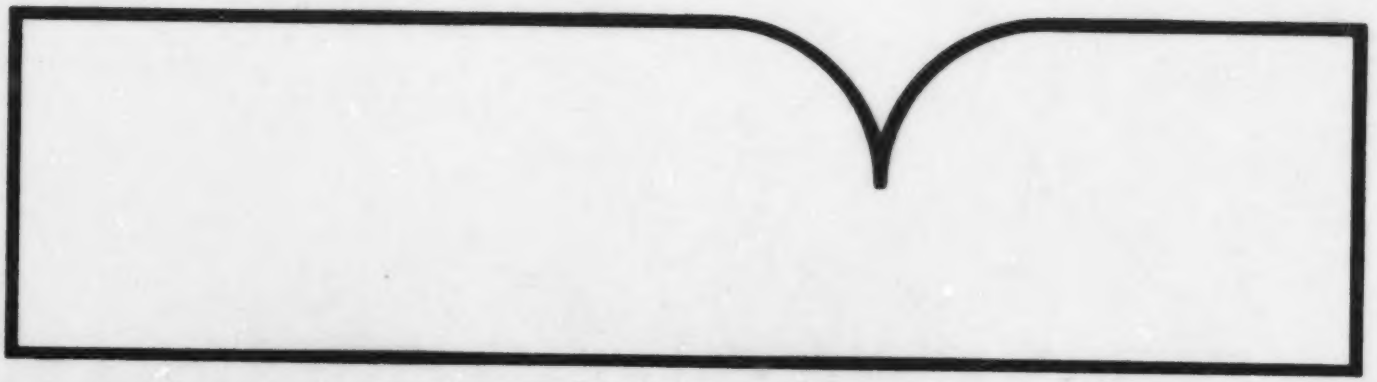

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16. ABSTRACT

We have developed a variety of non-invasive and other techniques to study effects of inhaled pollutants on the lung. In the area of airway mechanics we have developed and applied a diameter gauge to make continuous measurements of large airways caliber. The gauge provides an electrical output. We have also developed and validated rapid methods for non-invasively measuring respiratory mechanics using forced random noise excitation at the mouth. The resulting respiratory impedance data are applied to appropriate models to obtain values for parameters such as "central" and "peripheral" airways resistance.

In the area of repiratory epithelial function we have developed a non-traumatic technique to measure transepithelial potential difference across respiratory (nasal and airways) epithelium. We have also measured tracheal epithelial permeability in vivo, demonstrating increased permeability and decreased permselectivity in guinea pigs exposed to 4 ppm, 1 ppm and 0.3 ppm O_3.

In the area of pulmonary vasculature, we have developed a rapid non-invasive multi-gas rebreathing technique to measure lung water and used it to develop an O_3 - induced pulmonary canine model of delayed pulmonary edema using 1 ppm O_3.

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NEW APPROACHES TO QUANTITATING THE PULMONARY EFFECTS OF INHALED POLLUTANTS

by

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Major emphasis in this investigation was placed on the development of new methods and approaches to the study of pollutant effects on the lung. Particular stress was laid on procedures which might be applied to human studies.

F. G. Hueter, Ph.D.
Director,
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INTRODUCTION

Major emphasis in these investigations was placed on development of new methods and approaches to the study of pollutant effects on the lung. Particular stress was laid on procedures which might be applied to human studies. Three major areas are discussed:

A. Respiratory Mechanics
B. Pulmonary Microcirculation and Lung Water
C. Respiratory Epithelial Permeability and Airways Hyperreactivity

Respiratory mechanics studies focused on development of the technique of forced oscillations. It has been possible to develop an apparatus capable of making the basic pressure and flow measurements in spontaneously breathing humans as young as 3 years of age, and to develop algorithms to process the impedence versus frequency data so as to derive physiologically meaningful parameters. We will describe these models and algorithms in section 1, the instrumentation for forced random noise excitation of the respiratory system in section 2, the results of several experimental studies in humans and animals using this technique in section 3. In section 4 we describe the development of a device capable of providing continuous direct measurements of airway caliber which is not related to the random noise technique.

1. MODELS AND ALGORITHMS FOR RANDOM NOISE STUDIES:

In this program we were concerned with developing models and algorithms for interpreting the respiratory impedence spectrum. Several well accepted models of mechanical behavior have been described in the literature, and our work is based on these models. Our efforts in this area have been focused on the development of algorithms for estimating parameters in these various models. We have evaluated both mathematically rigorous and empirical approaches.

We have used regression analysis to define mathematically rigorous algorithms for defining parameter values that minimize the sum-squared-difference between the experimental impedence and the model's impedence. Since, for the simple series resistance, inertance, and the compliance model, the impedence is a linear function of all model parameters, we used linear regression analysis to derive equations for these three parameters as a function of experimental impedence data. In normal dogs we have shown that respiratory resistance and compliance calculated in this way correlated with corresponding parameters measured during tidal breathing although the forced excitation parameters were consistently lower. We believe this difference is due to some frequency dependence that does not affect the very low frequency tidal breathing measurement, but does affect the high frequency forced oscillation measurements.

In more complicated models with parallel compartments, the impedance is a nonlinear function of the model parameters. Thus, iterative techniques must be used in estimating parameter values. These iterative approaches require considerably more computer time than linear regression analysis, and there are potential problems with parameter stability and interpretability. We have evaluated these factors using 4 iterative algorithms with each of 3 parallel compartment models. With data from 2 normal subjects, 2 asymtomatic cigarette smokers, and 3 patients with obstructive lung disease, we found that the combination of a 2 stage simplex algorithm with a 5 element model similar to that defined by Mead provided the most stable estimates and the best fit to the experimental data.

In a more recent study using 5 normal human subjects and 5 patients with obstructive lung disease, we showed that these 5 parameters can be interpreted as central resistance, central inertance, airway compliance, peripheral resistance and tissue compliance. In the patients, we obtained reliable estimates for all parameters except the tissue compliance, which we believe requires lower frequency data for reliable estimation. In the normal subjects, peripheral resistance was too small, to be measured. Also, in the 5 patients, the total respiratory inertance and compliance from the 3 element models were consistently low compared to similar values for normal subjects and to equivalent values in the 5 element model for these patients. This suggests that in subjects with obstructive lung disease the parameter values based on the 3 element model may be difficult to interpret.

With regard to empirical approaches for processing impedance data, we described an algorithm for fractionating total respiratory resistance into central and peripheral components. This algorithm is based on the Mead model and it uses 2 linear regression analysis to relate the slope of the resistance versus frequency curve on log scales to the fraction of resistance in the periphery. Although this algorithm has some limitations, in normal dogs it assigned an average of 40% of the total resistance to the periphery. After correcting for the resistance of the endotracheal tube, the average percentage was 33%, a value that compares very favorably with others in the literature which range from 31 to 36%. In dogs studied with an added external resistance, this algorithm assigned an average of 85% of the added resistance to the central component. In sixteen 3-5 year old children, the empirical algorithm assigned an average of 53% of the total resistance to the peripheral component. In general, the fraction assigned to the periphery was higher in the 3 year olds than in the older children. These findings are also consistent with those in the literature.

Other empirically defined parameters that we have used are the average effective resistance at low and high frequencies, the ratio of the low and high frequency resistances, and the extrapolated resistance at 1 Hz. These parameters appear to provide a sensitive but arbitrary measurement of mechanical inhomogeneity.

In summary, several approaches for processing impedance data to obtain estimates of various resistance, compliance, and inertance parameters have been evaluated. Several of these algorithms provide estimates of the distribution of resistance between central and peripheral compartments. The algorithm based on nonlinear regression analysis appears to provide reasonable estimated, but it requires extensive computer time (on the order of one hour with a PDP 11/34).

The empirically based algorithm is efficient, but includes assumptions which may not be true always. We believe that a combination of these two methods in which the empirically based estimates are used to provide starting values and boundaries for the nonlinear regression algorithm may reduce the required computer time to a more reasonable value. Alternatively, the algorithm of Slutsky and Drazen or some modification of it may be useful. We believe that pursuing these options is extremely important because of a noninvasive, quick method for characterizing the peripheral airways would be a major contribution to the existing battery of pulmonary function tests.

2. DEVELOPMENT OF INSTRUMENTATION FOR RANDOM NOISE STUDIES:

This specific aim was concerned with developing the forced random noise technique for measuring the frequency dependence of respiratory impedance.

To provide random excitation with the appropriate bandwidth and statistical characteristics, we constructed a special electronic noise generator. Algorithms for collecting and processing random pressure and flow data in real time to obtain the impedance spectra were developed and tested. Also, the software to collect repeatedly a short pressure and flow record during a specific phase of the respiratory cycle was developed and tested. Methods for correcting the measured impedance for the effects of the bias tubing used in human studies and endotracheal tube in dog studies also were developed. Defining accurate corrections was not straightforward, but it was essential since errors in the corrections could lead to large errors in the corrected data.

In summary, we believe that we have developed the instrumentation and computer programs for collecting and correcting forced random noise impedance data in spontaneously breathing humans and intubated animals. This technique provides a rapid noninvasive measurement of data describing the dynamic mechanical properties of the respiratory system. In addition, the technique requires little subject cooperation and is designed so that data can be obtained during any phase of the respiratory cycle. In combination with algorithms for fractionating resistance, this approach will allow measurements defining the time course of regional mechanical changes in response to many physiological and pharmacological stimuli.

3. EXPERIMENTAL STUDIES WITH FORCED RANDOM NOISE:

This specific aim was concerned with experimental studies using the impedance spectrum and derived parameters to characterize physiological and pathological changes in mechanical function in both animals and humans.

Animal studies: In normal dogs we measured reasonable values of total respiratory resistance, compliance, and inertance using forced sinusoidal oscillations. In addition, changes in these values induced by adding external resistance, breathing He-O_2, or weighting the abdomen were consistent with predicted effects. Comparing data obtained with air and He-O_2 suggests that approximately 90% of the total respiratory inertance is due to the gas inertance while only 10% is attributed to the lung tissue and chest wall.

In dogs studied open and closed chested with forced oscillations, we found a mean decrease in resistance of 0.40 $cmH_2O/L/s$, which was 17% of the

closed chested resistance. This value was attributed to chest wall resistance, and it is fairly close to values predicted by a published regression equation. Appropriate calculations using reciprocals of opened and closed chested compliance, yielded a mean value of 0.177 L/cmH_2O. This value corresponds to 20% of the total respiratory elastance, and it also is fairly close to the value predicted for chest wall compliance from a published regression equation. There was no difference in the two inertances, so the chest wall was negligible inertance. This conclusion is consistent with that derived from the He-O_2 data mentioned earlier.

In dogs anesthetized with pentobarbital, we studied the effects of altering vagal tone on the forced oscillation parameters. Vagotomy produced no change in total respiratory resistance or compliance, but die produce an 8% decrease in total respiratory inertance. Electrical stimulation of both cut vagi adequate to cause cardiac arrest, increased resistance by 33%, increased inertance by 8%, and decreased compliance by 20%. The relatively small magnitude of the resistance changes compared to that reported by others, are attributed to the use of pentobarbital as an anesthesia, which attenuates vagally induced mechanical changes when compared to chloralose. With vagal stimulation we also noted that the effective resistance, that is, the real part of the impedance, became more frequency dependent suggesting the development of marked inhomogeneities in mechanical properties. Because of this, the fit between the data and the model was less good than that obtained without stimulation. These data were analyzed using our empirical algorithm for fractionating resistance. Vagal stimulation caused a larger mean change in peripheral resistance, 1.04 to 1.94 cmH_2O/L/s, than in central resistance, 2.12 to 2.68 cmH_2O/L/s. In addition we found that changes in peripheral resistance correlated with changes in compliance while changes in central resistance were uncorrelated with changes in compliance. This last finding supported the idea that peripheral resistance reflects the lung parenchyma.

We used the forced oscillatory technique to measure mechanical parameters in beagle dogs that had undergone a left pneumonectomy either at 6-10 weeks of age or at 1 year of age. The mean value of the total respiratory resistance was significantly higher for dogs treated at one year of age, 5.95 versus 4.06 cmH_2O/L/s. Similarly, the mean value of the total respiratory compliance was lower in the dogs treated at an older age, 0.0122 versus 0.0153 L/cmH_2O. Mean values of these parameters for the group treated at 6-10 weeks of age were not different from mean values for a control group of beagles. There were - significant differences between the control group and those treated at 1 year of age, although the differences were less marked than predicted by pneumonectomy and the subsequent hyperinflation of the residual lung. These findings suggest that following pneumonectomy, there are compensatory changes that are reflected in mechanical characteristics and that the compensation is more extensive when pneumonectomy is performed at a young age.

In dogs exposed to an aerosol containing papain, we found a 25% increase in total respiratory compliance measured by forced oscillations. Total respiratory resistance and inertance at FRC were unaltered by the papain exposure. However, resistance became more dependent on transrespiratory pressure after papain suggesting that the airways also became more compliant after papain exposure.

In several dogs we attempted to lodge 2 mm beads in the small airways to produce diffuse peripheral airway obstruction. We tried several techniques for placing beads including the use of a fiberoptic bronchoscope. In these procedures we essentially tried to blow the beads into the small airways, and obtaining the appropriate velocity proved very difficult. If the velocity was too low, the bead stuck in the walls of the large airways; if the velocity was too high, the beads produced hemorrhagic trauma in the parenchyma. Even when the beads were placed successfully in the small airways they were cleared rapidly by the movement of the mucous blanket.

In a recent study, we made forced random noise measurements both at the airway opening and at a site in a small airway using the retrograde catheter described by Macklem and Mead. Measurements were made after administering histamine either by aerosol or intravenous injection to alter mechanical characteristics. Measurements also were made at various transpulmonary pressures. This data is still being analyzed, but preliminary analysis indicates the peripheral resistance values are similar to those obtained by Macklem and Mead and that the compliance value obtained by subtracting total compliance and peripheral compliance is consistent with expected values for airway compliance.

Human Studies: In 12 normal adult subjects, total respiratory resistance, inertance, and compliance had mean values $\pm$ SD of 2.09 $\pm$ 0.44 $cmH_2O/L/s$, 0.0074 $\pm$ 0.0024 $cmH_2O/L/s^2$, and 0.0531 $\pm$ 0.031 L/cmH_2O, respectively. Resistance and inertance values were consistent with published values obtained by other methods. However, the compliance values were approximately 50% of generally accepted values, which we attributed to the inclusion of the effects of the contracting respiratory muscles. In 16 small children, 3-5 years, total respiratory resistance, and inertance were larger than in adults, while compliance was smaller. Mean values $\pm$ SD were 5.66 $\pm$ 0.49 $cmH_2O/L/s$, 0.0120 $\pm$ 0.0024 $cmH_2O/L/s^2$, and 0.00403 $\pm$ 0.00104 L/cmH_2O, respectively. These values were consistent with published data for this age group. Data obtained on 64 subjects ranging in age from 3 to 45 years suggests that total respiratory resistance decreases linearly with height and compliance increases linearly with height.

In normal adults, the mean coefficient of variation of repeated measurements of total respiratory resistance, compliance, and inertance were 7, 19, and 25% respectively. Corresponding values for 3-5 year old children were 12, 24, and 26% respectively. These data provide an estimate of measurement reproducibility.

In a study of 5 normal subjects, we compared total respiratory resistance, inertance, and compliance values obtained from forced random data obtained during a 1 sec. interval when inspiratory flow was maximum with corresponding values obtained during expiration. Resistance was 18% lower, inertance 26% higher, and compliance was 29% lower during inspiration than expiration. In addition repeated measurements on the same subject were less variable during inspiration than during expiration.

The real part of the impedance obtained by forced random excitation represents the effective resistance of the respiratory system. In normal adult subjects this usually was independent of frequency, although adult subjects resistance actually increased with frequency. In contrast, in adult patients with obstructive lung disease, effective resistance decreased markedly with frequency, and a similar, but less marked, effect was seen in young asymptomatic cigarette smokers. In normal young children, the frequency dependence in effective resistance was similar to that seen in adults with obstructive lung disease. As the age of the child increased, the observed frequency dependence became less marked, until at age 11 or 12 years resistance became independent of frequency. Thus, small children have lungs whose dynamic mechanical behavior is similar to the lungs of the adults with obstructive lung disease. This is attributed to the relatively smaller peripheral airways in young children.

In a study using 12 normal subjects, 15 asymptomatic cigarette smokers, and 8 patients with obstructive lung disease, we found significant differences between the mean values of all parameters for the patient group and those of either of the other two groups. Mean values for all parameters for the cigarette smokers were between those for the normal subjects and the patients. Significant differences between the mean values for the normals and the cigarette smokers existed for conductance normalized by height, resonant frequency, and the ratio of the 5-9 Hz resistance to the 15-19 Hz resistance.

In another study we attempted to look at the effect of smoking cessation on forced random noise parameters. Sixteen asymptomatic 21-26 year old male cigarette smokers with at least a 3 pack-year history were induced to quit smoking using 4 weekly group therapy sessions. Forced random noise, spirometric, plethysmographic, and single breath N_2 washout measurements were made at the beginning of the study and at monthly intervals for 3 months. Five subjects who stopped smoking completed the study. Mean changes in all parameters were in the direction of improved function, that is, resistance parameters decreased, forced expiratory spirometric timed volume and flow parameters increased, and closing volume and closing capacity decreased. Only the change in FEF 75% was statistically significant at the 0.05 level; however, changes in forced random noise resistance, FVC, FEV_1 closing capacity, and FRC were close to significance.

In another study, 12 normal subjects and 10 asymptomatic asthmatics were exposed to aerosols containing increased concentrations of methacholine. Airway resistance was measured plethysmographically and compared to 3 forced random noise resistance parameters. Airway resistance ranged from 0.7 to 13.4 $cmH_2O/L/s$. The 5-9 Hz resistance and the extrapolated resistance at 1 Hz correlated with the airway resistance with r-values of 0.91 and 0.93, respectively, and slopes of 0.51 and 1.56, respectively. For data where the airway resistance was less than 3.0 $cmH_2O/L/s$, the regression line relating the 5-9 Hz resistance had a slope of 1.03 and an intercept of 0.46. The intercept provides an estimate of lung tissue and chest wall resistance since it corresponds to the case where the airway resistance is zero. The slope of 1.03 indicates that changes in airway resistance are directly reflected in this forced random noise parameter. The 20-24 Hz resistance correlated poorly with airway resistance; in fact, as airway resistance increased, this parameter increased slightly and then became almost independent of airway resistance. These data were consistent with a model containing a parameter for central and peripheral resistance where methacholine exposure increased the latter more than the former.

In the methacholine study we characterized dose-response curves for several forced random noise parameters by defining a threshold dose and the slope of the curve above this threshold. We considered parameters that reflected overall resistive properties, like the 5-9 Hz resistance, as well as parameters that reflected the degree of mechanical inhomogeneities, like the ratio of the low to high frequency resistance. Mean values for the threshold dose and the slope for all parameters were approximately an order of magnitude higher in the asthmatic subjects than they were in the normal subjects. Thus, it appears that methacholine challenge may result in marked mechanical inhomogeneities and that these can be characterized by the forced random noise techniques.

In summary, the total respiratory impedance spectrum has been measured in a wide assortment of experiments using dogs and humans. Values of total respiratory resistance, inertance, and compliance derived from these spectra are consistent with accepted values, and changes in these parameters induced by various manipulations agree with predicted effects. The reliability and interpretability of parameters characterizing central and peripheral resistance and those characterizing mechanical inhomogeneities is less certain. If these parameters prove to be as consistent as the total respiratory parameters, then the forced random noise technique can be used to characterize the time course of regional mechanical changes induced by a variety of stimuli.

4. INSTRUMENTATION AND EXPERIMENTAL STUDIES WITH THE AIRWAYS DIAMETER TRANSDUCER

In this program we were concerned with measuring the static dynamic characteristics of the airways, especially the trachea. We have studied isolated dog trachea and intact anesthetized dogs.

We measured the static and dynamic characteristics of the catheter tip diameter transducer to determine if they were suitable for measurements of airway diameter. The transducer was a useable diameter range of 5.3 to 24.3 mm. However, the relationship between diameter and the output electrical signal is markedly nonlinear due to geometrical factors, and so it requires special calibrations. Measurements of the transducer's dynamic characteristics indicate that it is adequate to 8 Hz.

The catheter tip diameter transducer has been used to measure diameter as a function of transmural pressure in 5 isolated trachea. Two aspects of measurements were made: one with one strut of the transducer on the membrane and one with two struts straddling the membrane. The curves exhibited hysteresis. In 3 trachea, curves obtained with the struts in the two positions were similar; in the other 2 trachea displacements were larger with the strut on the membrane under compressive pressures. This effect is attributed to variations in the amount of invagination exhibited by the membrane. Resting diameters averaged 1.6 cm. The curves were almost flat for both extremes of pressure with a relatively linear portion connecting these two plateaus. The threshold pressures were approximately 20 cmH_2O of compression and 10 cmH_2O of distension; the mean slope of the straight line segment was 0.02 cm/cmH_2O. This corresponds to a specific compliance of .013 cmH_2O^{-1}. A second set of studies yielded very similar values of specific compliance, and they agree with literature data. The catheter tip diameter transducer has suitable characteristics for measuring changes in airway diameter provided that the posterior membrane is not deformed markedly. This implies that the gauge can measure resting airway diameters, tidal changes in diameter, and diameter changes due to alteration of bronchomotor tone. Diameter measurements made during large pressure changes, for example, during a forced expiration, may be difficult to interpret.

Using the special plethysmograph for isolated trachea, we have collected oscillatory pressure and flow data from 6 trachea at frequencies from 0.5 to 12 Hz. These data were analyzed with 3 models to obtain quantitative parameters describing the viscoelastic properties of the trachea. The first model was a simple Voigt model with a linear elastic and viscous element in parallel. Mean estimates $\pm$ SD of these parameters were 8.2 $\pm$ 1.96 cmH_2O/cm and 0.132 $\pm$ 0.046 $cmH_2O/cm/s$, respectively. The second model was a pressure vessel model where all volume changes were absorbed by the membrane which was characterized by a complex parameter related to the complex Young's modulus and various geometrical factors. Mean estimates $\pm$ SD for the real part was 9.91 $\pm$ 2.96 cm/H_2O, and for the imaginary part it was 0.213 $\pm$ 0.12 $cm/s/cmH_2O$. The third model was from the literature, and it used an elastance and resistance parameter based on the assumption that the complete volume change occurred by membrane deformation. Mean estimated value $\pm$ SD for the elastance parameters were 18.0 $\pm$ 11.5 cmH_2O/cm and mean estimates $\pm$ SD for the resistance parameter was 0.01 $\pm$ 0.08 $cmH_2O/cm/s$. These values were similar to those obtained by Bobbears et al (1978). All 3 models suggested that the tracheal deformation is dominated by its elastic properties and that the viscous effects are unimportant at frequencies up to 12 Hz and maybe higher. The first model, the simple Voigt model, yielded the least variable parameters and appeared to provide the best fit to the data.

In intact anesthetized dogs we have used the diameter transducer to map the diameter of the airways. These data show that diameter increased monotonically as the gauge is moved toward the carina. Tidal variations in diameter are more pronounced near the carina suggesting that the trachea is more compliant in this region. With this transducer we have been able to record changes in diameter due to the administration of anesthesia, apnea, rebreathing expired gases, vagotomy, vagal stimulation, and inhaled histamine. Finally, we have noted spontaneously rhythmic contractions in the trachea that had periods ranging from 30 to 130s.

In summary these studies indicate that the catheter tip diameter transducer can be used to measure directly the resting diameter in the large airways and to measure dynamically the changes in airway diameter induced by a wide assortment of physiological and pharmacological stimuli. In studies where the trachea is not deformed grossly by large compressive pressures, the measured diameters are reliable. However, diameter measurements with large transmural pressures may be difficult to interpret quantitatively. In spite of this shortcoming the transducer will enable us to measure the magnitude and time course, of regional changes in bronchomotor tone in response to a wide assortment of stimuli.

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B. Pulmonary Microcirculation and Lung Water

The pulmonary circulation is a principal site of toxic effects of oxidant gases such as O_3 and NO_2 which are noted for their edemagenic effects. In lower concentrations, the formation of edema occurs only after a period of many hours after exposure. Studies of the pulmonary microcirculation in man generally require cardiac catheterization. However, various investigators have developed isotopic, computerized tomographic, thoracic impedance, multiple indicator dilution, and inert gas rebreathing techniques in an attempt to non-invasively estimate lung water content. The extravascular water content of the lung reflects the balance between fluid filtration into the lung interstitium and disposal of interstitial fluid via the pulmonary lymphatics. Fluid filtration is powered by the balance of the Starling forces across the microvasculature and its rate depends on the permeability of the vascular barrier to water and salt and to oncotically significant macromolecules. Thus, increased interstitial lung water is a relatively early sign of pathologic alteration of the Starling microvascular forces, the permeability properties of the capillary bed, and/or the efficacy of fluid disposal mechanisms. The development of more overt pulmonary edema involves alveolar space filling by interstitial fluid either via alveolar epithelial or bronchiolar epithelial pathways and represents a later phase of edemagenesis. The mechanisms underlying O_3-induced edema are poorly understood. There is little or no information on microvascular hemodynamic changes associated with O_3 exposure. Some evidence for increased permeability of the alveolar-capillary barrier to albumin exists and both alveolar type I cell and capillary endothelial cell morphologic changes have been noted.

We have developed and validated a rapid, non-invasive inert-gas rebreathing technique suitable for estimating lung water and other microvascular circulatory parameters in human subjects, and have studied the effects of O_3 and other injurious agents on the pulmonary circulation and on lung water movements in a canine model using more invasive techniques as well as inert-gas rebreathing.

This section describes: (1) testing and validation of the inert-gas rebreathing method; (2) application of the rebreathing method of O_3-induced pulmonary edema in a canine model and to studies of low concentrations of SO_2 in human subjects; (3) study of O_3 effects on responsiveness of the pulmonary arteriolar vasculature to the probe of alveolar hypoxia. Publications and reports resulting from this work are listed.

1. TESTING AND VALIDATION OF THE REBREATHING METHOD

A computer program was written in FORTRAN IV and was implemented on a PDP 11/34 computer. The program included several commonly used data analysis algorithms for rebreathing techniques that allowed for evaluation of these various methods (see validation section below). A giant calibrated syringe, 5-way valve, and pneumotachograph system was constructed.

The method was first evaluated in a group of intact, anesthetized mongrel dogs. In seven dogs, pulmonary edema was induced by oleic acid injection into the right ventricle. Six dogs served as normal controls. At the conclusion of the last series of rebreathing measurements, the animals were killed and the lungs removed for gravimetric measurement of total lung water. Values of rebreathing measurements of combined pulmonary tissue and capillary blood volume (VTPC) were compared to the autopsy determinations of total lung water. Values of VTPC were calculated using four commonly used data analysis algorithms. A good correlation between VTPC and autopsy total lung water ($r=0.90$) was obtained using the data analysis algorithm of Sackner et al. This algorithm uses the $C^{18}O$ intercept to determine time zero and all data to construct the alveolar disappearance curves. Mean VTPC of all dogs using this algorithm was 96% ± 14% of autopsy measurements total lung water. Additionally, not only was VTPC increased in the dogs with induced pulmonary edema, but the rebreathing methodology also allowed for measurements of other useful parameters of pulmonary function including oxygen consumption, D_LCO, and FRC during induced pulmonary edema. Thus, it appeared that the rebreathing method could be used to obtain meaningful results of multiple pulmonary parameters including pulmonary tissue volume, which represents total lung water, in states of heterogeneous lung injury. Also, the data analysis algorithm of Sackner et al resulted in the best correlation of VTPC with lung water with acceptable intrasubject variation.

The rebreathing method, using the Sackner algorithm, was then evaluated in an animal model of cardiogenic pulmonary edema. Cardiogenic edema was produced by inflating a balloon catheter in the left atrium of dogs and infusing varying amounts of normal saline intravenously. Twenty-one dogs were serially studied using this protocol. Again, the final rebreathing measurements of VTPC were compared to autopsy determinations of total lung water. Mild to severe degrees of pulmonary edema was produced (total lung water ranged from 7 to 28 ml/kg body weight). Again a high correlation ($r=0.95$) was obtained between rebreathing VTPC and autopsy total lung water measurements. Thus, rebreathing estimates of lung water appeared accurate in states of mild-to-severe cardiogenic pulmonary edema.

Twenty normal intact anesthetized dogs were then studied. Rebreathing measurements of pulmonary capillary blood flow (Qc) were compared to simultaneously obtained dye dilution cardiac outputs (Q_T). These studies showed a good correlation ($r=0.92$) between rebreathing measurements of Qc and Q_T. Mean Qc measurements were 94% ± 3% SEM of the Q_T values and similar to that found by others.

The rebreathing method assumes homogenous ventilation to volume, ventilation to perfusion, and perfusion to volume distributions. In order to further understand the effects of maldistribution of ventilation to perfusion (V_A/Qc) distribution within the lung, which would be present in the heterogenously

injured lung, we evaluated two different dog models of V_A/Qc imbalance. The first model involved the creation of two lung parenchymal compartments in which each compartment could be relatively well-defined experimentally. Two anatomically well-defined types of V_A/Qc imbalance were created by: (1) reversible occlusion of the right main pulmonary artery; and (2) obstruction of the left main bronchus. A second model of V_A/Qc imbalance was established by ventilating intact dogs with positive end expiratory pressure (PEEP). PEEP in dogs has been shown to create reproducible bimodal distributions of V_A/Qc ratios within the lungs. Rebreathing measurements of Qc were again compared to simultaneously obtained measurements of Q_T, and VTPC was again compared to autopsy measurements of total lung water. In the first model of V_A/Qc imbalance, no significant alterations in any computer rebreathing parameters occurred following acute occlusion of the right main pulmonary artery, as compared to data obtained after release of the occlusion. No significant differences between mean VTPC measurements and post-mortem lung water measurements was found with the artery occluded or unoccluded. Mean Qc was 87% of mean Q_T after and during release of the occlusion. Significant decreases of similar magnitude in all rebreathing parameters including D_LCO (46%), alveolar volume (42%), Qc (44%) and VTPC (53%) occurred after obstruction of the left main bronchus. The first set of experiments (unoccluded and occluded artery) demonstrated that alteration in distribution of pulmonary blood flow following the right main pulmonary artery obstruction caused no changes in computed rebreathing parameters except for a small (14%) decrease in D_LCO which can possibly be accounted for by a decrease in capillary blood volume caused by the vascular obstruction. The focal cessation in ventilation to one lung following obstruction of the left main bronchus caused parallel decreases in all rebreathing parameters. Thus, in this extreme instance, the observed changes in the rebreathing parameters were consistent with the presumed size of the large ventilation defect. Therefore, relatively large perfusion defects do not affect the measurements of rebreathing measurement of lung water while complete ventilation defects caused predictable parallel decreases in all rebreathing parameters. Rebreathing data in the dogs ventilated with 10 cmH_2O PEEP for a total of 180 min. showed the mean VTPC at 180 min. to be 149 ml 20 ml ISD and similar to the autopsy lung water content of 130 ml 209 SD ($p>0.1$). A significant increase in alveolar volume and decrease in Qc was noted 60 min after ventilation with 10 cmH_2O PEEP and remained altered for the entire study period. Thus, during the creation of V_A/Qc maldistribution with normal and high V_A/Qc compartments and decrease in Q_T by the use of PEEP, accurate measurements of VTPC could be obtained.

It is, however, impossible to accurately create well-defined graded V_A/Qc imbalances experimentally. We therefore utilized the experiments involving the reversible occlusion of the right main pulmonary artery and reversible obstruction of the main bronchus in order to validate a multi-compartmental computer simulation of rebreathing methodology that we have developed. Once validated, the model simulation could then be used to determine theoretical errors in rebreathing estimates of VTPC caused by graded maldistributions of alveolar volume, blood flow and other rebreathing parameters. We developed a multi-compartmental model simulation of rebreathing. All computer programs were written in FORTRAN IV and implemented in a PDP 11/34 computer. The model simulates a lung rebreathing a mixture of 10% He, 0.3% CO, and 1.0% C_2H_2 in a stepwise manner through a deadspace from a reservoir in a closed system. The following assumptions were made in the design of the model:

1. Gases mix instantaneously after inspiration and expiration.
2. The respiratory quotient is 1.
3. Qc is maintained constant during rebreathing and there is no recirculation.
4. Inspiration and expiration occur in a stepwise fashion.
5. Gas uptake in each compartment follow a single exponential.

For each compartment, values for the following parameters were entered: (1) Qc; (2) D_L; (3) lung gas volume; (4) VTPC; (5) fraction of inspired volume to each compartment; and (6) inspiratory and expiratory dead space. In addition values for soluble gas partition coefficients for blood and tissue, total inspiratory time, and inspired volume also were entered in the model.

The model's response was compared with experimental data described in the previous dog experiments in which two focal V_A/Qc areas were created by obstructing a main pulmonary artery and occluding a main bronchus. The model was verified in two ways. First, parameter values for Qc, D_L, VTPC and lung gas volume were taken from the unoccluded artery and unobstructed bronchus portions of the dog experiments and entered as inputs into a two-compartment model. Distribution of total ventilation, lung volume and Qc were assigned to the right and left lung compartments according to previously reported lung weight distributions. The model outputs were analyzed using the data analysis algorithm of Sackner, et al. The model outputs were then compared to the experimental parameters that had been used as inputs.

Second, using the same experimental input parameters, the model was manipulated in order to mimic the previously described major experimental interventions. That is, first the blood flow to the right lung compartment was set at zero and the entire blood flow was shunted to the left lung compartment simulating the experimental occlusion of the right main pulmonary artery. This model was also used to simulate the obstructed left main bronchus condition by setting the ventilation to the left lung at zero and decreasing the inspired volume by 50%. The apparatus dead space was increased to 140 ml during the left bronchus occlusion condition because of the slight increase in volume because of the use of a divided endotracheal tube in these experiments. The outputs of both of these manipulations were then compared and correlated to the experimental input parameters to further verify the model.

There was a high correlation between the experimental inputs for D_L, VTPC, and Qc and the model outputs. The outputs for VTPC were systematically slightly lower than the experimental input values by 8% and probably explained on the basis that the dead space of the model is empty on the first inspiration.

During the cessation of blood flow to the right compartment in the model, no significant changes in model outputs for Qc and VTPC occurred; this is similar to the findings in the dog studies. The regression equation and correlation coefficient of the model output versus model input for VTPC during the cessation of blood flow to the right compartment was $VTPC_{output} = 16 + 0.86\ VTPC_{input}(ml)$ ($r=0.97$). There was a mean decrease of 48% in the model output for VTPC as compared to the input value following cessation of ventilation to the left compartment which again is similar to the dog data.

Thus, the model outputs in all three conditions: (1) no V_A/Qc maldistribution; (2) focal high V_A/Qc defects; and (3) focal low V_A/Qc defects, accurately simulated the findings encountered during the actual dog experiments. The agreement between the actual experimental results in dogs and the model outputs suggests that despite the assumptions made in development of the model, the salient features represented in the model simulation appear to be a meaningful representation of the rebreathing system. We then evaluated the effects of graded maldistributions of D_L, Qc, VTPC, FRC and ventilation (V_E) using a two-compartment model. The numerical values for the input parameters were chosen to represent a normal adult, i.e., Qc of 3 L/min, FRC of 3 L, D_LCO of 30 ml/min x mmHg, inspired rebreathing volume of 1.2 L, and anatomic dead space of 150 ml. These values were initially divided equally between the two compartments. Again, model outputs were analyzed to derive values of rebreathing parameters of Qc, D_L, and VTPC using the Sackner algorithm. Since we were primarily interested in evaluating the effects of maldistribution of physiologic parameters on VTPC and Qc, only the output results of these two rebreathing variables are reported. Inspiratory and expiratory time was again set at one second each. Each physiologic parameter was then maldistributed between the two compartments and the % error in calculated VTPC and Qc calculated as described in the model theory section. Although the parameter in question was maldistributed between the two compartments the total amount of the parameter was kept constant. The results for uneven distribution for D_L, Qc, VTPC, FRC and V_E were computed. These data demonstrate that severe uneven distribution for D_L, Qc, VTPC, FRC produce little error (no greater than 3%) in calculated Qc. Severe distribution of VTPC and Qc also result in a small error in calculated VTPC. The greatest error, underestimate of 7%, occurred when all the lung water was present in one parenchymal compartment and absent in the other. Maldistribution of D_L, FRC and V_E caused reasonably large underestimates of VTPC only when severe extreme shifts in these parameters occurred. For example, when only 10% of D_L was present in one compartment, the underestimate of VTPC was 17.2%, and when 10% of FRC was present a 25.3% underestimate occurred. Severe unevenness of distribution of V_E (20% of ventilation going to one compartment) resulted in severe underestimates of both Qc and VTPC. This suggests and corroborates our findings in the animal studies that rebreathing estimates of VTPC are reasonably accurate in states of mild-to-moderate lung disease.

2. APPLICATION OF THE REBREATHING METHOD

Animal Studies

The edemagenic effect of ozone inhalation is well-established. In general these studies have relied on post-mortem examination and wet/dry weight ratios of the lungs of small rodents. Animal studies have also suggested that the edemagenic effect is delayed. Thus, in mice, Scheel et al found histologic evidence of edema and leukocyte migration into alveolar spaces 20 hours after exposure to 1 ppm O_3 for 4 hours. Mice sacrificed immediately after termination of the exposure showed alveolar capillary engorgement but no alveolar edema.

Although it is assumed that the edemagenic effects of O_3 involves increased permeability of the alveolar capillary membrane related to some primary oxidative action, sequential studies of O_3-induced edemagenesis in larger experimental animals do not appear to have been carried out. The rebreathing technique provides estimates of lung water in human subjects and large animals. We have shown that rebreathing estimates of lung water and pulmonary capillary blood flow are reliable in the presence of lung edema induced by infusion of oleic acid in dogs as well as in normal animals.

Using rebreathing methodology to make sequential measurements we wished to establish a canine model of O_3 induced parenchymal lung injury and examine the dose-response and time course of edemagenesis in intact animals. In addition we wished to validate the use of the rebreathing method for detection of O_3-induced lung edema since this technique would be useful in human studies during both rest and exercise.

Following a baseline (pre-exposure period) study period, animals were then continuously exposed to either 1.0 ppm O_3 (n=10) or 0.3 ppm O_3 (n=9) for 4 hours. Nine animals were exposed to only humidified warmed air using the same exposure set-up for 4 hours and served as controls. Following completion of the exposure period, the animals were placed back on the volume ventilator for 30 min. at the same tidal volume and frequency as in the pre-exposure period and restudied (30 min. post-exposure period). They were then extubated, allowed to awaken, and were then kept overnight in an animal laboratory facility. The next day, the animals were reanesthetized, intubated, and placed on a volume ventilator set at the same tidal volume and frequency as the previous day. After a 60-90 min. period of stabilization, another set of variables were measured (24 H post-exposure period). Four of the dogs who were exposed to 1.0 ppm O_3 were allowed to awaken after the 24 hours post-exposure study period, and were restudied, in similar fashion, the next day (48 hours post-exposure period) and then killed. Two animals exposed to 1.0 ppm O_3 were studied only at the pre-exposure period, 30 min. after exposure, and at the end of the 48 hour post-exposure period.

Statistical analysis was performed using a student t-test for paired and unpaired variables.

RESULTS

Control (Air Exposed) Dogs

No significant changes occurred in $PaCO_2$, PaO_2, or pHa either 30 min. or 24 hours after 4 hours of exposure to humidified air. Additionally, no changes in rebreathing variables including oxygen consumption (VO_2), diffusing capacity ($D_L CO$), pulmonary capillary blood flow (Qc), functional residual capacity (FRC), or combined pulmonary tissue and capillary blood volume (VTPC) occurred following this sham exposure. Average total wet minus dry lung weight was 120 g $\pm$ 10 g SEM and averaged 95% $\pm$ 1% SEM of the final VTPC measurement for all nine dogs in this group. Extravascular lung water to blood-free dry lung weight ratio was 4.10 $\pm$ .19 SEM which is in the normal range for our laboratory. No interstitial or alveolar edema was seen in the random lung sections by light microscopy.

Ozone Exposed Dogs

Dogs exposed to 0.3 ppm O_3 for four hours had a significant decrease in PaO_2 30 min. after exposure as compared to the pre-exposure value. PaO_2 was not significantly decreased at 24 hours after exposure. A significant decrease in pulmonary capillary blood flow (mean decrease of 33%) occurred 30 min. post-exposure and remained depressed 24 after exposure. No other significant changes in rebreathing parameters or arterior blood gases occurred in eight of the nine dogs studied. One dog developed marked pulmonary edema 24 hours after exposure to 0.3 ppm O_3 as evidenced by a wet to dry lung weight ratio of 6.19 and histologic evidence of alveolar edema. No changes in arterial blood gases or rebreathing parameters, except for a 22% decrease in pulmonary capillary blood flow, occurred 30 min. after exposure. Because of the severe edema 24 post-exposure, rebreathing parameters could be obtained. This animal was the only one in this present study who was not allowed to fully awaken prior to being kept overnight in the animal facility after exposure. Although we cannot be certain, the severe degree of lung injury that occurred may have been related to the suboptimal post-exposure care rather than secondary to the O_3 exposure itself.

In the remaining eight dogs in this group, the total wet minus dry lung weights averaged 146 $\pm$ 17 g SEM and was 103% $\pm$ 2% SEM of the final VTPC measurement. The mean wet to dry lung weight ratio was 4.48 $\pm$ 0.28 SEM which although higher was not significantly different from the air exposed group of dogs. No evidence of microscopic alveolar edema was found in these eight dogs and only one dog had evidence of interstitial edema with alveolar septal thickening and perivascular cuffing on light microscopic evaluation.

Dogs exposed to 1.0 ppm O_3 for four hours had significant ($p < .05$) decreases, as compared to the pre-exposure value, in both PaO_2 and pHa 30 min. after exposure which remained decreased 24 hours after exposure. However, six dogs studied 48 hours after exposure had no decreases in PaO_2 or pHa as compared to the pre-exposure value. No changes in $PaCO_2$ were noted at any time after exposure. A significant decrease in pulmonary capillary blood flow of 34 % occurred 30 min. post exposure and remained depressed 24 and also 48 hours after exposure. There was also a significant increase (mean increase of 32%) in VTPC 24 hours post-exposure. Four dogs were autopsied at this time. The mean wet minus dry lung weight was 247 g $\pm$.37 (SEM) and wet/dry lung weight ratio was 4.69 $\pm$ 0.37 (SEM) both significantly ($p < .05$) increased as compared to the values in the control air exposed group of dogs. Again, the wet minus dry lung weights were not significantly different from the final VTPC measurements in this group of dogs. All four dogs in this group had light microscopic evidence of interstitial edema and three of the four animals also demonstrated alveolar edema. Six dogs were studied 48 hous post-exposure to 1.0 ppm O_3 and the only significant change on any measured variable compared to pre-exposure values was a 34% decrease in Qc. Wet minus dry lung weight was 152 g $\pm$ 36 SEM and the wet to dry lung weight ratio was 4.52 $\pm$ 37 SEM, neither significantly different from the air exposed group. Histologic evaluation of the lungs of this group showed no evidence of edema.

In order to evaluate the possibility that the ventilatory pattern induced by phrenic nerve stimulation may have played a role in inducing pulmonary edema in the 1.0 ppm O_3 exposure group, five additional spontaneously breathing intubated animals were exposed to 1.0 ppm O_3 for four hours without phrenic nerve stimulation. These animals were kept lightly anesthetized with pentobarbital during the exposure. Because of the small number of animals in this group, no statistical analysis was performed. All of these dogs were killed after the 24 hour post-exposure study period. A small decrease (11%) in PaO_2 was noted 24 hours post-exposure. No changes in VTPC or Qc occurred. The wet minus dry lung weight was 143 g $\pm$ 20 g SEM and the wet/dry lung weight was 3.65 $\pm$ .07 SEM.

The present study has demonstrated that acute exposure to relatively low concentrations of O_3 (0.3 and 1.0 ppm O_3) resulted in adverse cardio-pulmonary effects in anesthetized, intubated dogs. Specifically, changes in PaO_2 and pulmonary capillary blood flow occurred shortly (30 min.) after exposure to 0.3 ppm O_3 and changes in pHa, PaO_2, and pulmonary capillary blood flow in the 1.0 ppm O_3 group and the alterations in pulmonary capillary blood flow in both groups and PaO_2 in the 1.0 ppm O_3 group persisted an additional day. Furthermore, the decrease in pulmonary capillary blood flow, as compared to the pre-exposure value in the 1.0 ppm O_3 group, persisted up to two days post-exposure. Pulmonary edema development, demonstrated by an increase in rebreathing measurements of lung tissue volume as well as in autopsy determinations of lung water content and in histological evidence of edema, occurred in the 1.0 ppm O_3 group of dogs but was delayed one day and was not present two days following ozone exposure. Sham exposure to air or exposure to a lower concentration of O_3 (0.3 ppm) produced no significant changes in lung tissue volume following exposure except for one dog in the 0.3 ppm O_3 group. These latter experimental groups suggest that the protocol used (i.e. repeated intubations and anesthesia; the inhalation exposure procedure; employment of recovery periods between measurements) did not cause the development of edema seen in the 1.0 ppm O_3 group.

Additionally, the final rebreathing measurements of lung tissue volume correlated well with the gravimetric measurements of total lung water as well as with histologic evaluation of lung sections, again supporting the accuracy of the rebreathing method for measuring lung water content in the presence of moderate lung injury.

We found no significant decreases in functional residual capacity following exposure to ozone at either 1.0 or 0.3 ppm in the present study. Although no significant decreases in rebreathing estimates of diffusing capacity for carbon monoxide were found, there was a mean decrease of 8% at 24 hours in the 0.3 ppm O_3 group, of 14% at 24 hours post-exposure and 20% 48 hours post-exposure in the 1.0 ppm group. Watanabe and co-workers evaluated changes in pulmonary function including end-expiratory lung volume and single-breath diffusing capacity for carbon monoxide in a group of cats exposed to 0.26, 0.5, and 1.0 ppm O_3 for an average of 4.6 hours. They found only a slight tendency for end-expiratory volume to increase following exposure to O_3. The frequency of reduction of 10% or more on two consecutive measurements were as follows: control animals, 0/3; 0.26 ppm O_3, 2/5; and 1.0 ppm O_3, 4/9. These changes appear similar to the data we found using a rebreathing method for measurement of D_LCO.

The extent to which O_3 directly affects the lung depends in large part on the amount of pollutant which penetrates to the gas-exchange regions of the lung. This fact has to be taken into account when evaluating the effects of a given exposure. It is known that conscious, spontaneously breathing dogs show an increase in respiratory frequency and a decrease in tidal volume after O_3 exposure, possibly mediated through vagal afferent pathways. In addition there is a significant (approximately 60-70%) uptake of ozone by the upper airways in dogs. In the present study, we have bypassed the upper respiratory tract by intubating the dogs with a Teflon® endotracheal tube. Using the data of Yokoyama and Frank we can assume that at least 80-85% of the inspired O_3 reached the lower airways and lung parenchyma. We also maintained respiratory rate constant during the exposure period by using transvenous phrenic nerve stimulation. This method has been shown to maintain adequate ventilation and stability of lobar ventilation over prolonged periods of time in anesthetized dogs. Although we did not measure tidal volume during the exposure period, the fact that $PaCO_2$ monitored during the exposure period remained unchanged, that respiratory frequency was maintained constant by electrophrenic stimulation, and that no alterations in FRC were noted following exposure, indicates adequate maintenance of alveolar ventilation during the exposure period. It is interesting to note that the five animals who were allowed to breathe spontaneously via a Teflon® endotracheal tube while exposed to 1.0 ppm O_3 for four hours did not exhibit changes in either lung tissue volume, pulmonary cpaillary blood flow, or increases in wet/dry lung weight ratios 24 hours post-exposure. Additionally, no changes in $PaCO_2$ or respiratory rate were noted during the exposure period in these animals. The pattern of inspiration produced by electrophrenic respiration is characterized by peak inspiratory flow approximately twice that seen in spontaneously breathing dogs. This suggests that the more rapid inspiratory flow may have altered the distribution or penetration of the inspired O_3 so as to exceed the threshold for a critical degree of parenchymal injury. This possible increase in exposure of the lower airways and parenchyma may account in part for the exaggerated effects of exercise plus O_3 exposure on pulmonary function in humans and for the impaired performance of athletes noted during periods of photochemical smog.

b. Human Studies

The multiple gas rebreathing method was adapted for use at the exposure chambers of the EPA Human Studies Division. The analog data from the mass spectrometer were converted to a digital form and stored on magnetic tape at the exposure facility and later analyzed using the DEC PDP 11/34 computer in the Division of Pulmonary Disease, UNC, employing the same algorithms as in the previous animal studies. The rebreathing method was first used in a study evaluating the effects of 0.75 ppm sulfur dioxide. Acceptable data were obtained in the control periods and no effect on rebreathing parameters was noted after SO_2 or sham exposure. The method is therefore operable and available for use in human pollutant exposure studies at rest or during exercise.

3. EFFECTS OF O_3 ON THE PULMONARY VASCULATURE

We had previously noted changes in D_LCO and pulmonary capillary blood flow following O_3 exposure in dogs (see previous discussion). To further explore these findings, we evaluated the effects of O_3 exposure on pulmonary hemodynamic parameters by studying the pulmonary vascular response to hypoxia following O_3 exposure in dogs. Additionally, evidence has been accumulating that prostaglandins may modulate the pulmonary vascular response to hypoxia. Furthermore, the unsaturated hydrocarbon backbone of prostaglandins and the products of arachidonic acid metabolism make these compounds theoretically subject to oxidation by ozone.

Thirty-four dogs were intubated, anesthetized, paralyzed, and ventilated. The ventilator was adjusted to maintain a $PaCO_2$ of 35-40 mmHg. Catheters were placed in appropriate vessels for measurement of pulmonary arterial and capillary wedge pressure and systemic aortic pressure. Cardiac output was measured using a thermal dilution technique. Pulmonary vascular resistance was calculated as mean pulmonary artery pressure minus mean wedge pressure divided by cardiac output. Ozone was produced in a manner similar to previous dog studies. Changes in arterial blood gases and pulmonary hemodynamic parameters were measured in response to 20 min. of hypoxia (F_IO_2=0.10) before (pre-exposure period) and after (post-exposure period) exposure to either 1.0 ppm O_3 (n=8), 4.0 ppm O_3 (n=8), or air alone (n=10). Another group of dogs (n=8) received an infusion of indomethacin following the pre-exposure period and were then exposed to air. The mean baseline data (breathing room air) was compared to the mean data collected during the 20 min. of hypoxia by a one-way analysis of variance. This study demonstrated a significant effect of indomethacin on the pulmonary circulation both at baseline as well as an augmentation of the pulmonary vascular response to alveolar hypoxia as compared to the air exposed group of dogs. Our results following ozone exposure are not as clear. Following 1.0 ppm O_3 exposure, for one hour, an increase in baseline pulmonary artery pressure and pulmonary vascular resistance was seen as well as an increase in pulmonary vascular resistance during 20 min. of hypoxia similar in magnitude to the changes seen in the indomethacin group and less than that seen in the air-exposure group. Following 4.0 ppm O_3 exposure, only significant increases in baseline pulmonary vascular resistance and pulmonary artery pressure was seen in comparison to pre-exposure values within this group and less than that following 1.0 ppm O_3 or indomethacin. However, when the changes in post-exposure hemodynamic parameters were compared to pre-exposure values only the indomethacin treatment group reached statistical significance when compared to the control group. These findings may be related in part to the large variability seen in the measurements. Although the findings are not definite, it seems that O_3 may have effects on the pulmonary circulation possibly mediated via arachidonic metabolism.

SUMMARY

These studies in dogs and humans indicate the following:

1) Multiple inert-gas rebreathing technology is readily applicable to human studies and has adequate precision to detect modest changes in Qc, VTPC or D_LCO -- all useful parameters of pulmonary microvascular function.

2) Anesthetised, intubated dogs develop delayed pulmonary edema 24 hours after exposure to 1.0 ppm O_3 for 4 hours using electrophrenic respiration. The edema regresses by 48 hours. However, decreased Qc was an earlier and more persistent effect. The decreased Qc was also a feature of exposure to 0.3 O_3 although a statistically significant increase in lung water was not found. These data suggest that inhalation of environmentally relevant concentrations of O_3 cause significant abnormalities in the pulmonary circulation and that one should explore the effect of elevation of pulmonary microvascular pressures in animals previously exposed to 0.3 ppm O_3 to see whether the combined stress results in pulmonary edema. This would be a relevant animal model for humans with cardiac disease and chronically increased pulmonary capillary pressure. The animal model also permits study of flow and composition of lung lymph which would provide information concerning the physiologic mechanisms underlying ozone-induced edema.

3) Ozone inhalation alters pulmonary vascular resistance, even in the absence of edema. This effect suggests pulmonary arteriolar constriction and raises the possibility that pulmonary vascular tone may be modulated by O_3-related chemical mediators. Arachidonate metabolites suggest themselves and the similarity of the O_3 and indomethacin effects on hypoxic responsiveness of the pulmonary vasculative supports this hypothesis. The effect of O_3 on arachidonate metabolism deserves further study.

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C. Respiratory Epithelial Permeability and Airways Hyperreactivity

Although the lung vasculature is a major target of toxic oxidants, acute airways effects are also well-established. These include smooth muscle constriction as evidenced by increased airways flow resistance, tachypnea, irritative cough and increased secretion. The physiologic mechanism underlying these effects may be stimulation of airway irritant receptors with a vagally mediated reflex arc causing smooth muscle constriction and hypersecretion while tachypnea and irritative cough are medullary effects of irritant receptor stimulation. A striking acute decrease in vital capacity seen in some human subjects exposed to environmentally relevant O_3 concentrations may also be related to neural reflexes rather than to altered lung mechanics although this has not been established. Of interest is the fact that although the observed changes airways revert to normal follwoing cessation of exposure, there is a "mencory" of the effect of the O_3 exposure. This is manifest in at least two ways. Firstly, the acute effect of a given, relatively brief, O_3 exposure on human airway resistance and vital capacity diminishes if the exposure is repeated daily over a four or five day period. Regaining of the original level of reactivity to O_3 requires a period of several days or a week without exposure. Secondly, even after regression of the acute O_3 effects on airways flow resistance, there is significant enhancement of the bronchoconstrictor response to challenge by pharmacologii agents like histamine. The latter phenomenon represents an induced state of bronchial "hyperreactivity" and is therefore an interesting example of a group of conditions associated with acquired bronchial hyperreactivity.

O_3-induced bronchial hyperreactivity was first described in guinea pigs and later in dogs. We wished to show whether this effect also occurred in human subjects, and if so, whether it could be elicited by bronchoconstrictors other than histamine. The reason for this is that histamine is a known stimulant of irritant receptors while acetylcholine (or its longer-acting analogues, methacholine or carbachol) is not.

We were also concerned with the mechanism whereby O_3 exposure induced bronchial hyperreactivity and we specifically considered the hypothesis that the exposure caused increases in airways epithelial permeability to aerosolized inhaled drugs, thus allowing them to penetrate into the airway wall in higher concentrations and thus produce greater effects on sub-epithelially located effector tissues (e.g., smooth muscle, irritant receptors, etc).

1. Human Studies:

a. O_3 exposure effect on airways reactivity to histamine and mecholyl aerosols. These studies were carried out at the EPA Clinical Studies facility principally by Dr. Hazucha with assistance from Dr. Bromberg and others. Normal, non-smoking volunteers were exposed to 0.6 ppm O_3 for two hours, or sham-exposed to filtered air. The acute effects of the exposure were monitored with spirometry and body plethysmography and revealed a spectrum of acute response to O_3. Two hours after cessation of exposure (time required to allow full regression of these acute effects), the airways response to increasing doses of inhaled aerosolized histamine was measured and dose-response curves plotted. The mean curve after O_3 was left-shifted as compared to the mean curve after air (sham exposure). By 24 hours after exposure, increased airways response to histamine could no longer be demonstrated. Essentially similar results were obtained with a different group of subjects using methacholine as the provocative agent.

With histamine there is a suggestion that the position of the dose-response curve prior to O_3 exposure (i.e., native reactivity of airways to histamine) is positively correlated with the intensity of the subsequent acute response to O_3. This was not clear in the methacholine experiment. The results suggest that the known variability among healthy subjects in acute response to O_3 may correlate with their intrinsic airways reactivity to histamine and may therefore involve airway irritant receptors. The lack of correlation with intrinsic airway responsiveness to methacholine does not act via irritant receptors. Furthermore, the post-O_3 "sensitization" phenomenon does not appear to depend on a bronchoconstrictor agent which itself operates through airways irritant receptor stimulation since methacholine was about as effective as histamine. These data confirm and extend some of the recent observations reported by H. Boushey, J. Nadel and their collegues. They are compatible with, but do not prove, the hypothesis of increased airways epithelial permeability induced by O_3 exposure.

b. Measurement of trans-epithial potential difference (PD) of respiratory epithelium. We have succeeded in developing an electrode system for human as well as animal use which give meaningful local PD measurements of respiratory epithelium in nose, trachea and bronchi. The local effect of drugs and chemicals introduced onto the mucosa on PD can also be measured. Since PD is the product of short-circuit current (attributable to active ion transports, especially that of Na^+) and to the electrical resistance of the epithelium, PD values only partially characterize the bioelectric properties of the epithelium. Further characterization presently requires <u>in vitro</u> studies. The results obtained using this technique in dogs and humans are described in papers listed at the end of this section.

2. Animal studies:

a. Conscious, spontaneously breathing guinea pigs were exposed to 1 ppm or to 4 ppm O_3 in an environmental chamber for three hours. The <u>in vivo</u> permeability of airways epithelium to three molecular probes (^{14}C-mannitol, M. Wt. 180 daltons; ^{3}H-dextran, M.Wt. 10,000 daltons; horseradish peroxidase, M.Wt. 40,000 daltons) was examined using a technique developed by R.C. Boucher and his associates. Permeability measurements were made immediately after exposure (1 and 4 ppm O_3), 1 day later (4 ppm O_3), 3 days later (1 ppm O_3) and 7 days later (4 ppm O_3). The results show sharply increased plasma appearance rates of all 3 probes, with the largest probes. This indicates a loss of selectivity as well as a general increase in permeability and suggests that movement of other protein molecules across the epithelium would be enhanced (e.g., antigens).

Study of the tight junctional apparatus at the apical margins of the epithelial cells from tracheas of guinea pigs exposed to 4 ppm O_3 appear to show marked disorganization and disappearance of the junctional strands. Further work is required to confirm this abnormality and to correlate with the permeability changes. The permeability changes after exposure to 4 ppm O_3 were still present at 1 day, but not at 7 days. The changes after 1 ppm O_3 were no longer present at 3 days. Thus, the permeability lesion appears to be reversible. Whether this is due to repair of injured cells or to shedding and replacement remains to be determined.

b. Acute studies of <u>in vivo</u> PD in guinea pigs and in dogs exposed to 4 ppm and to 1 ppm O_3 have failed to show a significant decrease. This result is surprising

but may be due to an increase in active ion transport paralleling increased permeability. Ussing chamber studies of tracheal epithelial membranes will be required to elucidate this point. Alternatively, it may be that the permeability increase involves creation principally of a small number of channels suitable for macromolecules and that the conductivity of the epithelium for small ions is not greatly altered. In vitro studies will be necessary to investigate this possibility.

c. We also have examined in vitro responsiveness to histamine of tracheal smooth muscle strips obtained from O_3-exposed and sham-exposed guinea pigs by an isotonic contraction technique. We found no significant difference in the ED_{50} value of 5×10^{-6} M histamine. These studies do not rule out the possibility of a direct O_3 effect on smooth muscle. Further studies using acetycholine, K^+, norepinephrine as well as isoproterenol and electrical field stimulation are needed to more fully characterize the properties of tracheal smooth muscle. Preferably the muscle should be prepared from the tracheas of animals which have demonstrated increased post-O_3 reactivity to aerosolized methacholine or histamine.

PUBLICATIONS

Journal Articles:

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